DATA EVALUATION RECORD

PRODIAMINE

Study Type: OCSPP 870.1300 [§82-4] Acute Inhalation Toxicity Study in Rats

EPA Contract No. EP-W-16-018
Task Assignment No. 31-2-016 (MRID 00149483)

Prepared for
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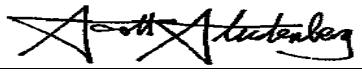
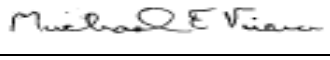
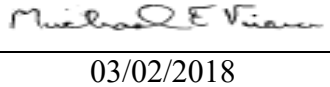
Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 02/15/2018
Quality Assurance: Michael E. Viana, Ph.D., DABT	Signature: Date:	 03/02/2018
Project Manager: Michael E. Viana, Ph.D., DABT	Signature: Date:	 03/02/2018

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. Contractor's role did not include establishing Agency policy.

EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature**: _____
RAB4, Health Effects Division (7509P) **Date:** **06-28-2018**
EPA Contract Officer Representative: Lori Brunsman **Signature**: _____
Science Information Mgmt. Branch, HED (7509P) **Date:** 06/28/2018
Template version 02/06

TXR#: 0056930

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity Study - Rats OCSPP 870.1300 [§81-3]; OECD 403.

PC CODE: 110201 **DP BARCODE:** D447498
TXR#: Not provided

TEST MATERIAL (PURITY): Prodiamine technical (purity not provided)

SYNONYMS: 2,6-dinitro-N^1,N^1-dipropyl-4-(trifluoromethyl)-1,3-benzenediamine

CITATION: Hardy, C.J., Jackson, G.C., Gregson, R.L., *et al*. (1985) Technical Prodiamine: Acute inhalation toxicity in rats, 4-hour exposure. Huntingdon Research Centre plc, Huntingdon, Cambridgeshire, England. Laboratory report No.: VCL 49/84839, March 5, 1985. MRID 00149483. Unpublished.

SPONSOR: Velsicol Chemical Corporation, 341 East Ohio Street, Chicago, IL.

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 00149483), groups of five Wistar (HC/CFHB) rats/sex/dose concentration were exposed to prodiamine (purity and Lot/Batch # not provided) by whole-body inhalation for a single 4-h exposure period at concentrations of 0 (clean air) or 0.256 mg/L. The rats were then observed for a total of 14 days and euthanized.

There were no effects of treatment on mortality, body weight or overall body weight gain, food consumption or water intake, lung weights, or microscopic examinations of the lungs, kidneys, and liver.

During exposure, closing/partial closing of the eyes, abnormal breathing, and abnormal body posture were noted in the prodiamine-exposed test animals. Findings were noted in some or all animals/sex as early as 15 min, and were observed in all males by 1.5 h and in all females by 3 h. Most treatment-related clinical effects resolved during the overnight retention period. Abnormal breathing was observed in a single animal/sex throughout the observation period (14 days), and sneezing was noted in a single male up to Day 10. Yellow staining of the fur was noted in all animals throughout the exposure period, but was resolved prior to the end of the observation period.

Body weight gain was decreased in the test animals for the Day 0-1 period in males (−0.4 g test vs. 3.0 g control) and females (−2.8 g test vs. −0.8 g control), respectively. Body weight gain values were similar between control and test animals by the Day 1-2 period, and for the overall Day 0-14 period. Minor decreases in food or water intake on Day 1 were not considered adverse.

Lung congestion was noted in two test males, with no additional effects noted in test females or any control animals. Congestion was considered a potential treatment-related effect.

As there was only a single treatment level (0.256 mg/L), systemic and/or portal-of-entry LOAEC estimates cannot be determined. The noted clinical effects and lung congestion are considered related to treatment, and indicate a portal-of-entry effect and a potential systemic effect.

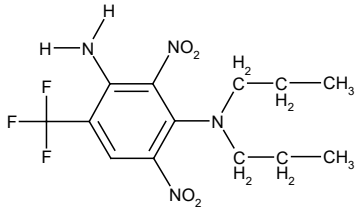
This study was conducted prior to the 870.1300 guideline and has a number of deficiencies. It is Acceptable as a limit test and satisfies the 870.1300 guideline for an acute inhalation toxicity study.

COMPLIANCE: A signed and dated GLP Compliance statement was provided attesting the study was conducted in compliance with 40 CFR Part 160 (1983) and OECD, ISBN 92-64-12367-9 (1982). Data Confidentiality and Quality Assurance statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Prodiamine technical

Description:	Yellow-gold powder
Lot/Batch #:	Not provided
Purity:	Not provided
CAS #:	29091-21-2
Structure:	

2. Vehicle and/or positive control: Clean air

3. Test animals

Species:	Rat	
Strain:	Wistar (HC/CFHB)	
Age/weight at exposure:	Approximately 7-9 weeks / males: 188-207 g; females: 185-210 g	
Source:	Hacking and Churchill Ltd. (Huntingdon, Cambridgeshire, England)	
Housing:	Five rats/sex/cage in suspended, polypropylene cage with wire-mesh top and floor.	
Diet:	Scientific Feeds LAD 1, *ad libitum*, except during exposure.	
Water:	Tap water, *ad libitum*, except during exposure.	
Environmental conditions:	**Temperature:**	22±1.0°C to 26±1.81°C
	Humidity:	50±5.4%
	Air changes:	Not provided
	Photoperiod:	Not provided
Acclimation period:	Not provided	

B. STUDY DESIGN

1. In-life dates: Start: July 18, 1984 End: August 1, 1984 (calculated by reviewers)

2. Animal assignment, dose rationale, and treatment: The rats were randomly (method of randomization not reported) assigned to two groups of five rats/sex/group (control and test).

A dose-selection rationale or a rationale for selection of the whole-body inhalation route were not provided. Although not stated explicitly, a target dose was not pre-selected. It was stated that the gear ratio of the generator mechanism was set to give the highest practical concentration of dust, and the exposure concentration was determined based on collected air samples.

After a 12-min equilibration period, a 4-h exposure period was conducted for the control or test group, followed by 60-min clearing period after stopping the generator prior to removal of the rats for examination. The rats were retained overnight in a ventilated cabinet, and returned to their regular cages the following day for the rest of the 14-day observation period.

3. Generation of the test atmosphere / chamber description: Diagrams of the Wright dust generator and the complete exposure system were provided as Figures 1 and 2, respectively, on pages 17 and 18 of MRID 00149483.

The Perspex whole-body exposure chambers were square with pyramidal tops, and an internal volume of 0.13 m^3. Animals (five/sex/treatment) were held in a stainless steel-mesh cage in individual compartments during the exposure period. The test atmosphere was introduced to the exposure chamber through a central port at the top of the chamber, and exited through openings at the bottom edge of the chamber.

A Wright dust generator was used to produce the test atmosphere by suspending the test material (from a compressed powder) in a stream of dry air. A portion of the test compound was packed in the dust generator with a hydraulic bench press with a force of 0.65 tons weight. The test atmosphere was passed from the generator through an aerosol neutralizer, to prevent electrostatic charge effects, to the top of the exposure chamber. The gear ratio of the generator was positioned to yield the highest practical dust concentration. The flow rate (measured at the generator outlet nozzle) was set at 25 L/min. Exposure was initiated by engaging the dust generator gearing and starting the motor.

Control animals were treated in a manner similar to the animals exposed to the test compound, but were exposed to clean air only.

Chamber air temperature and water vapor were determined at 30-min intervals during each exposure period. Relative humidity for the chamber was calculated from the temperature and water vapor content data. Although the mean (±SD) chamber air temperatures (26.5±0.16°C test vs. 26.4±0.33°C control) and relative humidity levels (24±4.9% test vs. 22±4.8% control) were similar to control values, both parameter values were outside the ranges of the current Guideline recommendations (22±2 or 3°C and 30-70%, respectively).

Test atmosphere concentration: The achieved analytical concentrations are presented in Table 1. Five air samples were collected (i.e., 30 min, 50 min, 1 h 55 min, 2 h 55 min, and 3 h 48 min) from the exposure chamber during the 4-h exposure period. Each air sample was drawn through a glass-fiber filter in a filter holder at a rate of 4 L/min. Additional information regarding the sampling procedure were not provided. Air samples were analyzed to determine the actual test compound concentrations during exposure. The concentration in the air samples was determined with a gas chromatography (GC) method. Details of the analytical method were included as Appendix 1 on pages 28 and 29 of MRID 00149493.

TABLE 1. Mass concentrations in test air samples [a]

Sample time	Dose group (mg/L)	
	Control	Test
0 h 30 min	-	0.253
0 h 50 min	-	0.266
1 h 55 min	-	0.251
2 h 55 min	-	0.271
3 h 48 min	-	0.239
Mean±SD	-	0.256±0.0127

a Data were obtained from page 13 of MRID 00149483; n=10.

The mean (±SD) of prodiamine aerosol concentrations delivered to the test group were determined to be 0.256±0.0127, with a coefficient of variation (CV) of approximately 5%. This variability was stated to be acceptable, and was within the current guidelines of ±20% for a dry aerosol.

Test atmosphere temporal stability and homogeneity: The temporal stability and homogeneity of the generated prodiamine atmosphere was assessed during the sample collections that were made to determine the atmosphere concentration.

Mean temporal homogeneity for the test chamber over the 4-h exposure period was approximately 5%. These homogeneity results are considered acceptable.

Particle size distribution: Two additional air samples were collected with an Andersen mini sampler, at a sampling rate of 1.4 L/min and sample times of 1 h 30 min and 3 h 30 min during the 4-h exposure period. The Andersen sampler consists of four stages and a filter that differentiates particle sizes from >5.5 µm aerodynamic diameter (a.d.) [Stage 1] to <0.3 µm a.d. [filter]. The collected material was weighed, and the particle size distribution of prodiamine was determined.

The determined particle size distribution of aerosolized prodiamine during the 4-h exposure period is shown in Table 2. Approximately 30% and 60% of the aerosolized test material was >5.5 µm during the two sampling periods, and trapped at Stage 1. The remainder of the aerosolized material (<5.5 µm) accounted for 68.5% and 43.0% of the sampled test aerosol, and was considered respirable (mean 56%).

TABLE 2. Particle size distribution of aerosolized prodiamine during a 4-h exposure period. [a]

Sample time	Stage	Particle size (µm)	Prodiamine in stage (mg)	% of Total	% Respirable
Determination #1					
1 h 30 min	1	>5.5	1.505	31.5	68.5
	2	3.5-5.5	0.700	14.6	
	3	2.0-3.5	1.040	21.7	
	4	0.3-2.0	1.055	22.0	
	Filter	<0.3	0.485	10.1	
	Total		4.785	99.9	
Determination #2					
3 h 30 min	1	>5.5	2.15	57.0	43.0
	2	3.5-5.5	0.68	18.0	
	3	2.0-3.5	0.42	11.1	
	4	0.3-2.0	0.40	10.6	
	Filter	<0.3	0.12	3.2	
	Total		3.77	99.9	

a Data were obtained from Table 1 on page 19 of MRID 00149483.

4. Statistics: Statistical analyses were not determined.

C. METHODS / OBSERVATIONS

1. Mortality and clinical observations: All animals were observed continuously during the exposure period, and twice daily during the observation period, for treatment-related clinical signs.

2. Body weight: Body weights were determined daily on all animals from the day of receipt until the end of the 14-day observation period (scheduled euthanasia).

3. Food and water consumption: Cage food and water intakes were determined daily from the day after receipt until the end of the 14-day observation period (scheduled euthanasia). Mean daily intake/rat was determined for food and water.

4. Sacrifice and pathology: After completion of the 14-day observation period, animals were euthanized by exsanguination after pentobarbitone anesthesia (i.p.), and subjected to a detailed macroscopic examination. The lungs were removed, weighed, and infused and fixed in 10% buffered formalin. Portions of the liver and kidneys also were collected and fixed in 10% buffered formalin. All fixed tissues were processed routinely, stained with hematoxylin and eosin, and examined microscopically.

II. RESULTS

A. OBSERVATIONS

1. Mortality: All rats survived to scheduled euthanasia after Day 14. Accordingly, the estimated LC_{50} was considered >0.256 mg/L.

2. Clinical signs: During exposure, closing/partial closing of the eyes, abnormal breathing, and abnormal body posture were noted in the treated group. Findings were noted in some or

all animals/sex as early as 15 min, and were observed in all males by 1.5 h and in all females by 3 h. Yellow staining of the fur was noted in all animals throughout the exposure period. No clinical signs were observed in control animals during the exposure period.

Most treatment-related clinical effects resolved during the overnight retention period. Abnormal breathing was observed in a single animal/sex throughout the observation period (14 days), and sneezing was noted in a single male up to Day 10. Yellow staining of the fur and/or tail persisted through the initial part of the observation period, but was absent by Days 8 and 9 in males and females, respectively.

B. BODY WEIGHTS AND BODY WEIGHT GAINS: Body weight and body weight gain data are presented in Table 3. Although not analyzed statistically, there were no apparent, treatment-related effects on daily body weights. Body weight gain (calculated by the reviewers) was decreased in the test animals for the Day 0-1 period in males (−0.4 g test vs. 3.0 g control) and females (−2.8 g test vs. −0.8 g control), respectively. Body weight gain values were similar between control and test animals for the Day 1-2 and Day 0-14 periods.

TABLE 3. Mean (±SD) body weights and body weight gains (g) in rats after exposure to prodiamine for a 4-h period. [a]

Day	Dose (mg/L)	
	Control (0)	Test (0.256 mg/L)
Male		
0 (Exposure)	193.6±5.77	196.4±5.98
1	196.6±8.99	196.0±7.35
2	207.4±8.65	204.2±8.20
3	213.6±9.21	212.0±10.20
7	246.6±11.70	243.6±11.59
14	297.4±14.98	296.6±17.42
BWG 0-1 [b]	3.0	−0.4
BWG 1-2 [b]	10.8	8.2
BWG 0-14 [b]	103.8	100.2
Female		
0 (Exposure)	195.8±3.11	199.0±9.03
1	195.0±3.87	196.2±9.20
2	200.0±2.12	200.8±10.94
3	202.4±2.70	205.8±8.14
7	212.2±3.19	216.8±10.57
14	227.8±4.97	237.0±15.05
BWG 0-1 [b]	−0.8	−2.8
BWG 1-2 [b]	5.0	4.6
BWG 0-14 [b]	32.0	38.0

a Data were obtained from Table 4 on pages 22-23 of MRID 00149483; n=5/sex/dose. Standard deviations calculated by the reviewers from individual data.

b Body weight gains calculated by the reviewers from individual data.

C. FOOD AND WATER CONSUMPTION: Food consumption was decreased in males and females, and water intake was decreased in males, by approximately 20% for Day 1 of the observation period, but all values for the remainder of the observation period were similar to control (not evaluated statistically). No differences were noted for water intake in female rats.

D. SACRIFICE AND PATHOLOGY (GENERAL TOXICITY GROUP)

1. **Gross pathology:** Red or gray areas in the lungs were noted in both control and test animals (two test males and two test females vs. two control males and one control female). Lung congestion was noted in two test males, with no additional effects noted in test females or any control animals. Congestion was considered a potential treatment-related effect.

2. **Organ weights:** Organ weight data are presented in Table 4. Lung weights were the only organ weight determined. No treatment-related differences in lung weights or lung to body weight ratios were noted (data not evaluated statistically).

TABLE 4. Mean (±SD) body weights and body weight gains (g) in rats after exposure to prodiamine for a 4-h period. [a]

Parameter	Dose (mg/L)	
	Control (0)	Test (0.256)
Male		
Terminal body weight (g) [b]	297.4±14.98	296.6±17.42
Lung wt, abs (g) [c]	1.246±0.061	1.310±0.067
Lung wt, relative to BW (%)	0.418±0.015	0.442±0.041
Female		
Terminal body weight (g) [b]	227.8±4.97	237.0±15.05
Lung wt, abs (g) [c]	1.164±0.091	1.136±0.103
Lung wt, relative to BW (%)	0.512±0.035	0.480±0.035

a Data were obtained from Table 7 on page 26 of MRID 00149483; n=5/sex/dose.
b Identical to Day 14 body weights.
c Mean (±SD) calculated by the reviewers from individual data.

3. **Microscopic pathology:** No treatment-related microscopic effects were observed in the lungs after acute, 4-h inhalation exposure to prodiamine. Aggregations of alveolar macrophages were noted in one control female and two test females. One of these test females also presented with diffuse pneumonitis. Other minor, non-treatment-related microscopic effects noted included mineral foci at the renal corticomedullary junction (four control and three test females), single foci of renal cortical tubular basophilia (one control female and one test male and female), and a hepatic parenchymal focus of inflammatory cells in a single control female.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS: A discussion or conclusion by the study authors was not provided.

B. REVIEWER COMMENTS: There were no effects of treatment on mortality, body weight or overall body weight gain, food consumption and water intake, lung weights, or microscopic examinations of the lungs, kidneys, and liver.

The present study was conducted in 1984, and although it was stated that it was conducted in accordance with GLP regulations, no QA statement was provided. Although not stated explicitly, the study was conducted in the manner of a preliminary, exploratory study. As

this MRID was reviewed against the OCSPP 870.1300 and OECD 403 guidelines, numerous deficiencies were noted.

During exposure, closing/partial closing of the eyes, abnormal breathing, and abnormal body posture were noted in the test compound-exposed test animals. Findings were noted in some or all animals/sex as early as 15 min, and were observed in all males by 1.5 h and in all females by 3 h. Most treatment-related clinical effects resolved during the overnight retention period. Abnormal breathing was observed in a single animal/sex throughout the observation period (14 days), and sneezing was noted in a single male up to Day 10. Yellow staining of the fur was noted in all animals throughout the exposure period, but was resolved prior to the end of the observation period.

Body weight gain was decreased in the test animals for the Day 0-1 period in males (−0.4 g test vs. 3.0 g control) and females (−2.8 g test vs. −0.8 g control), respectively. Body weight gain values were similar between control and test animals by the Day 1-2 period, and for the overall Day 0-14 period. Minor decreases in food or water intake on Day 1 were not considered adverse.

Congestion was noted in two test males, with no additional effects noted in test females or any control animals. Congestion was considered a potential treatment-related effect.

As there was only a single treatment level (0.256 mg/L), systemic and/or portal-of-entry LOAEC estimates cannot be determined. The noted clinical effects and lung congestion are considered related to treatment, and indicate a portal-of-entry effect and a potential systemic effect.

C. STUDY DEFICIENCIES: The following study deficiencies were noted:

- A Certificate of Analysis for the test material was not provided.
- Air change, photoperiod, and acclimation period details were not provided.
- A dose rationale was not provided.
- Only a single dose was tested, and the dose was only determined based on measurements conducted during the exposure period. Guideline recommendations require a minimum of three dose levels or a limit dose evaluation.
- Chamber temperature and relative humidity recommendations were not met.
- No QA evaluation was provided.